PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P706 PC00	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/DK 03/00827	International filing date *(day/month/year)* 02.12.2003	Priority date *(day/month/year)* 03.12.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant AARHUS UNIVERSITET et al.		

1. This International preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21.06.2004	26.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Favre, N Telephone No. +49 89 2399-7363

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK 03/00827

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-56 as originally filed

Claims, Numbers

1-46 received on 11.04.2005 with letter of 08.04.2005

Drawings, Sheets

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 46 and claims 23, 24 and 27 (partially)

   because:

   ☒ the said international application, or the said claims Nos. 46 (with regard to industrial applicability), relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 23, 24 and 27 (partially)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-46
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-46
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-45
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item I

Basis of the report

1. Sequence listing pages 1-7 filed with the letter of 18.03.2004 do not form part of the application (Rule 13ter.1(f) PCT).

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claim 46 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(I) PCT).

2. Moreover, as indicated in the international search report, claims 23, 24 and 27 have only been partially searched. In accordance with Rule 66.1(e) PCT, the present report has only been established for the subject-matter in respect of which an international search report has been drawn (Rule 70.2(d) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. For the assessment of the present claim 46 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognise as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

2. Document D1 (WO-A-02/06460) discloses, e.g. page 27, lines 15-25, a method for determining a predisposition for a manifestation of an immune system related disease (see page 4, line 34 - page 5, line 4) comprising determining in a biological sample the presence or absence of a polymorphism within the amino acid sequence of MASP-2.
The applicant argued that D1 does not specifically teach a method for determining a predisposition for a manifestation of an immune system related disease wherein the presence or absence of a polymorphism within the amino acid sequence of MASP-2 is assessed.
In view of the facts that D1 teaches the importance of MASP-2 against infection and teaches that a deficiency in MASP-2 renders an individual susceptible to infection (page 33, lines 12-18), it is however considered that the skilled person would consider the method of detecting presence or absence of a polymorphism within the amino acid sequence of MASP-2 taught in D1 at least in this context, i.e. for the determination of a predisposition of an individual for a manifestation of an immune system related disease.
It is therefore considered that the subject-matter of independent claim 1 differs from the teachings of D1 in that the polymorphism is to be found at the N-terminal of MASP-2 (CUB1, EGF, CUB2, CCP1 and CCP2 domains).
In view of the applicant's argument, the problem underlying the present invention could be seen in the provision of an effective method for determining a predisposition for a manifestation of an immune system related disease in an individual.
According to the applicant's arguments, this problem is solved by identifying a polymorphism within the N-terminal of MASP-2.
It has been argued that the application as filed only identifies one single polymorphism which is associated with a predisposition for a manifestation of a

immune system related disease in an individual, namely a change from Asp to Gly in position 105 of SEQ ID NO:1 or 2. Consequently, it has been submitted that the above-mentioned problem (the provision of a method for determining a predisposition for a manifestation of an immune system related disease in an individual) could not be considered to be solved by independent claim 1 over its whole scope.
In response to this argument, the applicant has experimentally demonstrated that another mutation (insertion within the EGF domain) within the N-terminal of MASP-2 also leads to a predisposition for a manifestation of an immune system related disease in an individual.
In view of this additional experimental data, and in the absence of any founded doubt that other mutations within the N-terminal of MASP-2 will not lead to a predisposition for a manifestation of an immune system related disease in an individual, the subject-matter of independent claim 1 is considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

2.1 Dependent claims 2-7 further define specific embodiments of the novel and inventive method of claim 1.
Dependent claims 2-7 are hence also considered to meet the requirements of Articles 33(2) and 33(3) PCT.

3. The arguments presented herein-above with regard to the subject-matter of claims 1-7, also apply for the subject-matter of claims 8-22, which differ from said claims 1-7 and that they refer to SEQ ID NO:3 (DNA sequence) and not to an amino acid sequence (SEQ ID NO:1).
The subject-matter of dependent claims 8-22 is thus also considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

4. The arguments presented herein-above with regard to the subject-matter of claims 1-7, also apply for the subject-matter of claims 23-32.
The subject-matter of dependent claims 23-32 is thus also considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

5. Documents D1 and D3 (Molecular Immunology, 1998, **35**(6-7):409) disclose antibodies recognising MASP-2. The particular epitope recited in claim 32 (wild type epitope) is however not specifically mentioned or taught in these documents.
The subject-matter of independent claim 33 is therefore considered to be novel in the sense of Article 33(2)PCT.
Moreover, since these particular antibodies can be used in order to perform the novel and inventive methods of e.g. claims 1-7, the subject-matter of independent claim 33 is considered to be inventive in the sense of Article 33(3) PCT.

6. Finally, in view of the arguments presented herein-above with regard to the subject-matter of claims 1-7, the subject-matter of claims 34-46 is also considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

Claims

1. A method for determining a predisposition for a manifestation of an immune system related disease in an individual comprising determining in a biological sample isolated from said individual the presence or absence of a polymorphism within the amino acid sequence of the MASP-2 protein as identified in SEQ ID NO: 1 and/or within the amino acid sequence of the MAp-19 protein as identified in SEQ ID NO: 2, said polymorphism being a substitution, deletion and/or addition of least one amino acid residue.

2. The method of claim 1, wherein the polymorphism a substitution, deletion and/or addition of at least one amino acid within a fragment of MASP-2 consisting of CUB1, EGF, CUB2, CCP1 and CCP2 domains.

3. The method of claim 1, wherein the polymorphism being a substitution, deletion and/or addition of at least one amino acid within a fragment of MASP-2 and/or MAp-19 consisting of CUB1, EGF, CUB2 domains.

4. The method of claim 1, wherein the polymorphism being a substitution, deletion and/or addition of at least one amino acid within a fragment of MASP-2 and/or MAp-19 consisting of CUB1.

5. The method according to any of the preceding claims, wherein the polymorphism being a substitution, deletion and/or addition of at least one amino acid located within amino acid residues from position 80 to position 120 according to the amino acid sequences set forth in SEQ ID NO: 1 or 2.

6. The method according to any of the preceding claims, wherein the polymorphism being a substitution or deletion of Asp in position 105 according to the amino acid sequences set forth in SEQ ID NO: 1 or 2.

7. The method of claim 6, wherein the substitution being Asp→Gly.

8. A method for determining a predisposition for a manifestation of an immune system related disease comprising determining the presence or absence of

polymorphism within the coding DNA sequence (SEQ ID NO: 3) of the human MASP-2 gene, said polymorphism being a substitution, addition or delition of at least one nucleotide within said coding DNA sequence.

9. The method of claim 8, wherein the DNA sequence comprising the polymorphism is a coding nucleic acid sequence for the proteins as defined in any of the claims 1-7.

10. The method of claim 8, wherein the polymorphism being a single nucleotide substitution/mutation A→G in position 359 corresponding to the sequence set forth in SEQ ID NO: 3.

11. The method according to any of the claims 1-10, wherein the polymorphism is determined by isolating the MASP-2 and/or MAp-19 proteins from a biological sample collected from an individual and ascertaining the substitution/mutation in the amino acid sequence of said proteins by a method selected from the group comprising mass-spectroscopy methods, such as MALDI-TOF mass-spectroscopy, protein sequencing methods or immunoassays.

12. The method according to any of the claims 1-11 further comprising isolating the MBL-MASP or ficolin-MASP complexes from a biological sample collected from an individual and examining the activity of said complexes, said activity being determined as an ability the complexes to activate the C4 complement.

13. The method according to any of the claims 1-12 further comprising examining the protein composition of MBL or ficolin complexes in a biological sample collected from an individual.

14. The method according to any of the claims 1-13, wherein the predisposition to a manifestation of an immune system related disease is determined by the absence of the MASP-2 (SEQ ID NO: 1) and/or MAp19 (SEQ ID NO: 2) proteins in the MBL or ficolin complexes.

15. The method according to claims 8-10, wherein the presence or absence of the polymorphism is detected by hybridising a probe to a target nucleic acid

sequence comprising at least position 359 according to the SEQ ID NO: 3 or SEQ ID NO: 4 or the corresponding position of the complementary strand.

16. The method according to claim 15, wherein the probe is bound to a detectable label.

17. The method according to claim 16, wherein the label is selected from a group comprising fluorescent reporter groups, enzyme tags, chemiluminescent groups or radioisotopes.

18. The method according to claim 15, comprising the use of a capture probe for capturing a target nucleic acid sequence.

19. The method according to any of the preceding claims 15-18, comprising amplification of a nucleotide sequence comprising the polymorphism.

20. The method according to claim 19, wherein amplification comprises use of a primer pair comprising SEQ ID NO: 5 and 6 or SEQ ID NO: 7 and 8.

21. The method according to claim 8, wherein the presence or absence of the polymorphism is detected by using isolation of a target nucleic acid from an individual said target nucleic acid comprising at least position 359 according to the sequence set forth in the SEQ ID NO: 3 or the corresponding position of the complementary strand and sequencing of said isolated target nucleic acid.

22. The method according to any of the claims 8-21, further comprising assessing the alleles at nucleotide no. 359 according to the sequence set forth in SEQ ID NO: 3 in a target nucleotide sequence corresponding to SEQ ID NO: 3 or the complementary strand.

23. An isolated oligonucleotide comprising at least 10 contiguous nucleotides of SEQ ID NO: 3 or the corresponding complementary strand, said nucleic acid sequence comprising the G allele in position 359 or the corresponding allele of the complementary strand.

24. The isolated oligonucleotide according to claim 23, comprising at least 15 contiguous nucleotides, more preferably at least 20 nucleotides.

25. An isolated polynucleotide sequence encoding the MASP-2 polypeptide having Gly at positon 105 according to amino acid sequence set forth in SEQ ID NO: 1.

26. An isolated polynucleotide sequence encoding the MAp-19 polypeptide having Gly at positon 105 according to amino acid sequence set forth in SEQ ID NO: 2.

27. The isolated oligonucleotide or polynucleotide sequence according to any of the claims 23-26, wherein the nucleotides are selected from RNA, DNA, LNA, PNA monomers or chemically modified nucleotides capable of hybridising to a target sequence.

28. An isolated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 1 or a fragment thereof, said polypeptide or said fragment comprising Gly in position 105 according to said sequence.

29. An isolated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2 or a fragment thereof, said polypeptide or said fragment comprising Gly in position 105 according to said sequence.

30. An isolated peptide fragment having a size in a range from 5 to 160 amino acids derived from the amino acid sequence set forth in SEQ ID NO: 1 comprising at least 5 amino acid contiguous sequence, said sequence corresponding to amino acid residues 100-105, 101-106, 102-107, 103-108, 104-109 and/or 105-110 of the sequence set forth in SEQ ID NO: 1.

31. An isolated peptide fragment having a size in a range from 5 to 160 amino acids derived from the amino acid sequence set forth in SEQ ID NO: 1 comprising at least 5 amino acid contiguous sequence, said sequence corresponding to amino acid residues 100-105, 101-106, 102-107, 103-108, 104-109 and/or 105-110 of the sequence set forth in SEQ ID NO: 1, wherein Gly in position 105 of said sequence is substituted for Asp.

32. An isolated antibody capable of recognition of the MASP-2 and/or MAp-19 polypeptides or fragments thereof, said polypeptides and fragments comprising Gly in position 105 according to the SEQ ID NOS: 1 or 2, by selectively binding to an epitope comprising said Gly or selectively binding to an epitope created within said polypeptides or said fragments due to mutation of Asp→Gly in position 105 according to SEQ ID NOS:1 or 2.

33. An isolated antibody capable of recognition of the MASP-2 and MAp-19 polypeptides or fragments thereof by selectively binding to an epitope comprising Asp corresponding to position 105 of the sequence set forth in SEQ ID NOS: 1 or 2.

34. A kit for predicting an increased risk of a subject of developing an immunologic disease comprising at least one probe comprising a oligonucleotide sequence as defined by any of the claims 23-27 and/or at least one probe comprising at least one antibody as defined by claims 32 and 33, or a fragment of said antibody.

35. The kit according to claim 34, wherein the probe is linked to a detectable label.

36. The kit according to any of the claims 34-35, further comprising a set of primers for amplifying a region of the human MASP-2 gene said region comprising position 359 according to SEQ ID NO: 3 or the corresponding complementary strand.

37. A gene therapy vector for the treating pathologic conditions associated with low activity of MBL-pathway in a subject carrying the G allele in the position corresponding to nucleotide no 359 of the sequence identified in SEQ ID NO: 3.

38. The gene therapy vector of claim 37, said vector comprising the sequence set forth in SEQ ID NO: 3, or a fragment thereof operably linked to a promoter sequence capable of directing the in vivo expression of MASP-2 encoded by SEQ ID NO: 3.

39. A gene therapy vector for the treating therapeutic conditions associated with pathologically high activity of the MBL-pathway, said vector comprising the

nucleotide sequence identified as SEQ ID NO: 3, said sequence having substitution A→G in position 359, said sequence operably linked to a promoter sequence capable of directing the in vivo expression of MASP-2 having glycine residue in position 105 according the sequence set forth in SEQ ID NO: 1.

40. Use of a polypeptide comprising any of the amino acid sequences set forth in SEQ ID NOS: 1 or 2, or fragments thereof, said polypeptides or said fragments comprising Gly in position 105 of said sequences, for production of a medicament for the inhibition of activity of the lectin-complement pathway.

41. Use of a peptide fragment according to claim 40 for inhibition of activity of the lectin-complement pathway.

42. Use of a polypeptide comprising any of the amino acid sequences set forth in SEQ ID NOS: 1 or 2, or fragments thereof, said polypeptides or said fragments comprising the glycine residue in position 105 of said sequences for the manufacture of a medicament for treatment of therapeutic conditions associated with pathologically high activity of the lectin-complement pathway.

43. Use of an oligonucleotide and/or polynucleotide as defined in any of the claims 25-28 for the manufacture of a medicament for treatment of therapeutic conditions associated with pathologically high activity of the lectin-complement pathway.

44. Use of an antibody as defined in claim 33 or a fragment thereof for the manufacture of a medicament for treatment of therapeutic conditions associated with pathologically high activity of the lectin-complement pathway.

45. The use according to claim 42-44, wherein the therapeutic conditions associated with pathologically high activity of MBL-complement pathway being an inflammatory disease, ischemia, apoptosis or atherosclerosis.

46. A method of treatment of an individual having a predisposition to a manifestation of an immune system related disease comprising

I) identification a mutation in the MASP-2 gene of said individual and

administering to said individual an effective amount of a polypeptide comprising SEQ ID NO:1 and/or polypeptide comprising SEQ ID NO:2.

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